REMARKS

Claims 1-22 are pending in the present case. These claims stand rejected under 35 U.S.C. § 112, first and second paragraphs. Claims 1-4, 6, 9, 12, and 13 stand further rejected under 35 U.S.C. § 102. Each of these rejections is addressed below.

Support for Amendments

Applicants have amended the specification to update the status of all cited U.S. patent applications and to a correct typographical error that occurs in Figure 6 and the legends to Figures 5 and 6. Support for correction of this typographical error is found in the specification, for example, at page 15, lines 14, 15, 20, 21, and 23, and page 28, lines 6-7. An equivalent correction to the typographical error has been made to Figure 6. Typographical errors have also been corrected at pages 4, 13, and 24.

In addition, claims 1 and 8-22 have been amended. Claim 1 has been amended to include the limitations of claims 3, 4, and 7 (which claims are now canceled), and to further specify that the claim is directed to a library of scaffold-based proteins that include members having the ability to bind compounds as a result of randomizing at least three loops. These amendments find support in the specification at page 19, lines 17-18, and page 3, line 9 - page 4, line 9, respectively. The amendment to claim 8 finds support in the specification, for example, at page 14, line 20 - page 15, line 2. The remaining claim amendments merely change claim dependencies or bring the language of the

dependent claims into conformity with the language of amended claim 1.

No new matter is added by any of these amendments.

Applicants reserve the right to pursue all canceled subject matter in this or any currently pending or future related application.

Objection to the Drawings

As required, Applicants submit herewith new Figures 1-12. These drawings correct the errors recited in the recent Form PTO-948. This objection may be withdrawn.

Rejections under 35 U.S.C. § 112, second paragraph

Claims 1-22 stand rejected, under 35 U.S.C. § 112, second paragraph, as being indefinite. This rejection is based first on the assertion that it is "unclear what sequences are considered randomized since the claims do not contain a template sequence with which to compare." The Office goes on to suggest that "[A]ddition of a template sequence with which to determine if a loop was randomized would clarify this matter." As recommended by the Office, Applicants have amended the claims to specify that the claimed proteins are scaffold-based proteins and that the scaffold is the tenth module of the human fibronectin type III domain (^{10}Fn3), the template sequence of which was known in the art at the time of filing (see the specification at page 7, lines 18-22). In view of this amendment, this basis for the rejection may be withdrawn.

In addition, the term "randomized" is rejected as unclear based on the assertion that this term is more appropriately used when describing a library of proteins. In response to this rejection, the Office is first directed to Applicants' specification at page 8, lines 4-5, where the term "randomized" is defined:

> By "randomized" is meant including one or more amino acid alterations relative to a template sequence.

In view of this definition, Applicants believe the term to be definite. In addition, Applicants note that the claims are now directed to a library of proteins. This basis for the rejection may therefore also be withdrawn.

The final basis for the indefiniteness rejection focuses on the claim term "corresponding naturally-occurring fibronectin." This term has been removed from the claims, and this basis for the rejection is now moot.

Rejections under 35 U.S.C. § 112, first paragraph

Claims 1-22 stand further rejected, under 35 U.S.C. § 112, first paragraph on the basis that the specification does not provide a written description for the claimed invention, nor does the specification reasonably provide enablement for that invention. As applied to the present claims, this rejection is respectfully traversed.

I. Written Description

Claims 1-22 first stand rejected as failing to provide an adequate written

description for the claimed invention. This rejection turns on the assertion that the claims cover "any protein containing at least one loop and that binds any compound not bound by a "corresponding" fibronectin. Proteins encompassed by the claims may have any binding activity or enzymatic activity and almost any structure (in addition to the "at least one randomized loop")."

The Office, citing the Guidelines for Examination of Patent Applications under the Written Description Requirement, states that "the written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice, reduction to drawings, or by disclosure of relevant, identifying characteristics (i.e., structure or other physical and/or chemical properties), by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus." This standard is met for the present claims.

Claims 1 and 8-22, as amended, are directed to a "library" of proteins that specifically include a scaffold derived from "the tenth module of the human fibronectin type III domain (^{10}Fn3)." The claims further specify that these proteins have "at least three randomized loops" and are "characterized by their ability to bind to compounds that are not bound by said human fibronectin type III domain" and where the compound binding ability results from the "randomization of said at least three loops." These claims

satisfy the written description requirement.

In particular, actual reduction to practice of a library of proteins covered by these claims is described in the present specification at pages 21-27, and is specifically described at page 26, lines 15-20. The proteins of this library include the tenth module of the human fibronectin type III domain and have three randomized loops (BC, DE, and FG). The specification also indicates that a combination library that included three-randomized loop, two-randomized loop, and one-randomized loop species was used for the selection of proteins that bound to TNF-α, a protein not bound by human ^{10}Fn3 (see specification at pages 27-28).

Moreover, the current claims directed to this library of proteins and the specification supporting these claims do, in fact, include "relevant, identifying characteristics" of library members that are both structural and functional in nature. In particular, the specification states and the claims require that the library proteins include a scaffold derived from the tenth module of the human fibronectin type III domain. This domain possesses a well known scaffold structure (as shown, for example, in Figure 3), and also possesses three loops that can be randomized, thereby providing the functional characteristic of binding activity to non-fibronectin target proteins. As stated by Applicants at page 3, line 3 - page 4, line 9 of the present specification:

> The present invention provides a new family of proteins capable of evolving to bind any compound of interest. These proteins, which make use of a fibronectin or fibronectin-like scaffold, function in a manner characteristic of natural or engineered antibodies (that is, polyclonal, monoclonal, or single-chain

antibodies) and, in addition, possess structural advantages. Specifically, the structure of these antibody mimics has been designed for optimal folding, stability, and solubility, even under conditions which normally lead to the loss of structure and function in antibodies.

These antibody mimics may be utilized for the purpose of designing proteins which are capable of binding to virtually any compound (for example, any protein) of interest. In particular, the fibronectin-based molecules described herein may be used as scaffolds which are subjected to directed evolution designed to randomize one or more of the three fibronectin loops which are analogous to the complementarity-determining regions (CDRs) of an antibody variable region. Such a directed evolution approach results in the production of antibody-like molecules with high affinities for antigens of interest. In addition, the scaffolds described herein may be used to display defined exposed loops (for example, loops previously randomized and selected on the basis of antigen binding) in order to direct the evolution of molecules that bind to such introduced loops. A selection of this type may be carried out to identify recognition molecules for any individual CDR-like loop or, alternatively, for the recognition of two or all three CDR-like loops combined into a non-linear epitope.

This description is reiterated and expanded in the specification at page 12, line 13 - page 13, line 14, where it states:

The novel antibody mimics described herein have been designed to be superior both to antibody-derived fragments and to non-antibody frameworks, for example, those frameworks described above.

The major advantage of these antibody mimics over antibody fragments is structural. These scaffolds are derived from whole, stable, and soluble structural modules found in human body fluid proteins. Consequently, they exhibit better folding and thermostability properties than antibody fragments, whose creation involves the removal of parts of the antibody native fold, often exposing amino acid residues that, in an intact antibody, would be buried in a hydrophobic environment, such as an interface between variable and constant domains. Exposure of such hydrophobic residues to solvent increases the likelihood of aggregation.

In addition, the antibody mimics described herein have no disulfide bonds, which have been reported to retard or prevent proper folding of antibody fragments under certain conditions. Since the present scaffolds do not rely on disulfides for native fold stability, they are stable under reducing conditions, unlike antibodies and their fragments which unravel upon disulfide bond

breakdown.

Moreover, these fibronectin-based scaffolds provide the functional advantages of antibody molecules. In particular, despite the fact that the ^{10}Fn3 module is not an immunoglobulin, its overall fold is close to that of the variable region of the IgG heavy chain (Figure 2), making it possible to display the three fibronectin loops analogous to CDRs in relative orientations similar to those of native antibodies. Because of this structure, the present antibody mimics possess antigen binding properties that are similar in nature and affinity to those of antibodies, and a loop randomization and shuffling strategy may be employed in vitro that is similar to the process of affinity maturation of antibodies in vivo.

In view of the above, it is clear that the present specification sets forth a written description for the presently claimed libraries of proteins possessing the structural and functional characteristics of the tenth module of the human fibronectin type III domain having randomized loops that result in binding to non-fibronectin targets. The written description rejection of claims 1 and 8-22 should be withdrawn.

II. Enablement

Claims 1-22 stand further rejected, under 35 U.S.C. § 112, first paragraph on the basis that the "specification, while being enabling for a library of proteins, does not reasonably provide enablement for an individual undefined variant protein of a fibronectin type III domain" (emphasis added). As applied to the currently claimed invention, this rejection may be withdrawn.

The present claims are directed to the library of proteins indicated by the Office to be enabled. Methods for producing this library, the members of which include a human ^{10}Fn3 scaffold having three randomized loops, are taught in the specification at pages 21-

27. And methods for using the library, as acknowledged by the Office, are taught throughout the specification, for example, at page 13, lines 15-18. The currently claimed invention is enabled by the present specification, and this basis for the § 112, first paragraph rejection may be withdrawn.

Rejections under 35 U.S.C. § 102

Claims 1-4, 6, 9, 12, and 13 stand further rejected, under 35 U.S.C. § 102(a), as being anticipated by Koide et al. (J. Mol. Biol. 284:1141-1151 (1998)). This rejection is respectfully traversed.

To support a rejection of a claim under § 102, a single prior art reference must describe all of the elements and limitations of the rejected claim. *Scripps Clinic & Research Foundation v. Genentech, Inc.*, 927 F.2d 1565, 18 U.S.P.Q.2d 1001, 18 U.S.P.Q.2d 1896 (Fed. Cir. 1991). Koide does not meet this standard in supporting a rejection of the present claims.

As an initial matter, Applicants note that only the abstract of this reference was available (on the internet) prior to the filing date of Applicants' parent application, filed December 10, 1998. Applicants provide herewith the publication citation available from the MEDLINE database, demonstrating that the full journal article was not published until December 11, 1998. Thus, only the Koide abstract may be properly cited against the current application under 35 U.S.C. § 102(a).

In addition, Applicants note that claim 1 and all dependent claims now include the limitation of original claim 7, a claim determined by the Office to be free of the prior art rejections. Indeed, the currently claimed invention was not disclosed in either the abstract or the full Koide reference. The current claims require a library of proteins, the members of which include human ^{10}Fn3 scaffold-based proteins having at least three randomized loops that result in compound binding activity. Koide does not teach such a library. The Koide abstract does not disclose using as a scaffold either the tenth domain or a human species of Fn3. Furthermore, the Koide publication merely discloses proteins that include a fibronectin type III domain containing mutations at particular positions in two loops and that bind a ubiquitin target protein with those two loops. Koide does not teach or suggest that at least three loops of the protein could or should be randomized. In fact, if anything, Koide teaches that issues of protein structure and stability counter-indicate randomization of Fn3 sequences outside of the two BC and FG loops.[1] Koide therefore does not anticipate the current claims, and this basis for the § 102 rejection may be withdrawn.

Claims 1-4 and 12 also stand rejected, under 35 U.S.C. § 102(b), as being anticipated by Campbell et al. (Structure 2:333-337 (1994)). As applied to the current claims, this rejection is also respectfully traversed.

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[1] Applicants note that Koide also indicates that the N-terminal tail, adjacent to the BC and FG loops in the three-dimensional Fn3 structure, might also be amenable to mutation; this sequence is not a loop structure.

Again, Applicants note that current claim 1 and all dependent claims now include the limitation of original claim 7, a claim determined by the Office to be free of this rejection. Campbell fails to disclose a library of proteins, the members of which include human fibronectin type III scaffolds having at least three randomized loops. Campbell instead discloses only naturally-occurring modules from fibronectin and related proteins. This basis for the § 102 rejection may also be withdrawn.

Conclusion

Applicants submit that this case is now in condition for allowance, and such action is respectfully requested.

Applicants note that a Supplemental Information Disclosure Statement was filed on September 6, 2001 (after the mailing of the present Action). Applicants request that this Statement be reviewed by the Examiner, and the accompanying Form PTO-1449 be initialed and returned with the next Action.

Enclosed is a petition to extend the period for replying for three months, to and including January 17, 2002.

If there are any additional charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: 17 January 2002

Karen L. Elbing, Ph.D.
Reg. No. 35,238

Clark & Elbing LLP
176 Federal Street
Boston, MA 02110
Telephone: 617-428-0200
Facsimile: 617-428-7045

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MARKED UP VERSION TO SHOW AMENDMENTS MADE

In the Specification:

Replace the first paragraph on page 1 (lines 7-8) with the following amended paragraph:

This application claims the benefit of the filing date of co-pending application, U.S.S.N. 60/111,737, filed December 10, 1998, now abandoned.

Replace the second full paragraph on page 4 (lines 17-24) with the following amended paragraph:

Any of the fibronectin type [II] III domain-containing proteins described herein may be formulated as part of a fusion protein (for example, a fusion protein which further includes an immunoglobulin F_c domain, a complement protein, a toxin protein, or an albumin protein). In addition, any of the fibronectin type III domain proteins may be covalently bound to a nucleic acid (for example, an RNA), and the nucleic acid may encode the protein. Moreover, the protein may be a multimer, or, particularly if it lacks an integrin-binding motif, it may be formulated in a physiologically-acceptable carrier.

Replace the final partial paragraph on page 4 (line 25) with the following amended partial paragraph:

The present invention also [includes] features proteins that include a

Replace the fourth paragraph on page 11 (lines 10-14) with the following amended paragraph:

FIGURE 5 is a photograph showing the structural similarities between a

^{10}Fn3 domain and 15 related proteins, including fibronectins, tenascins, collagens, and undulin. In this photograph, the regions are labeled as follows: constant, dark blue; conserved, light blue; neutral, white; variable, red; and [RGB] RGD integrin-binding motif (variable), yellow.

Replace the fifth paragraph on page 11 (lines 15-19) with the following amended paragraph:

FIGURE 6 is a photograph showing space filling models of fibronectin III modules 9 and 10, in each of two different orientations. The two modules and the integrin binding loop [(RGB)] (RGD) are labeled. In this figure, blue indicates positively charged residues, red indicates negatively charged residues, and white indicates uncharged residues.

Replace the fourth paragraph on page 13 (lines 20-24) with the following amended paragraph:

The antibody mimics of the present invention are based on the structure of a fibronectin module of type III (Fn3), a common domain found in mammalian blood and structural proteins. This domain occurs more than 400 times in the protein sequence database and has been estimated to occur in 2% of the proteins sequenced to date, including fibronectins, [tenscin] tenascin, intracellular cytoskeletal

Replace the second partial paragraph on page 19 (lines 14-24) with the following amended partial paragraph:

The antibody mimics described herein may be used in any technique for evolving new or improved binding proteins. In one particular example, the target of binding is immobilized on a solid support, such as a column resin or microtiter plate well, and the target contacted with a library of candidate scaffold-based binding proteins. Such a library may consist of ^{10}Fn3 clones constructed from the wild type ^{10}Fn3 scaffold through randomization of the sequence and/or the length of the ^{10}Fn3 CDR-like loops. If desired, this library may be an RNA-protein

fusion library generated, for example, by the techniques described in Szostak et al., U.S.S.N. 09/007,005, now U.S. Patent No. 6,258,558 B1, and 09/247,190, now U.S. Patent No. 6,261,804 B1; Szostak et al., WO98/31700; and Roberts & Szostak, Proc. Natl. Acad. Sci. USA (1997) vol. 94, p. 12297-12302. Alternatively, it may be a DNA-protein library (for example, as described in

Replace the first partial paragraph on page 20 (lines 1-9) with the following amended partial paragraph:

Lohse, DNA-Protein Fusions and Uses Thereof, U.S.S.N. 60/110,549, filed December 2, 1998 and 09/453,190, filed December 2, 1999). The fusion library is incubated with the immobilized target, the support is washed to remove non-specific binders, and the tightest binders are eluted under very stringent conditions and subjected to PCR to recover the sequence information or to create a new library of binders which may be used to repeat the selection process, with or without further mutagenesis of the sequence. A number of rounds of selection may be performed until binders of sufficient affinity for the antigen are obtained.

Replace the last paragraph on page 24 (lines 18-20) with the following amended paragraph:

Unispl-s ([spint] splint oligonucleotide used to ligate mRNA to the puromycin-containing linker, described by Roberts et al, 1997, supra): 5'-TTTTTTTTTNAGCGGATGC-3' (SEQ ID NO: 13)

Replace the second paragraph on page 25 (lines 10-22) with the following amended paragraph:

Next, each of the double-stranded fragments was transformed into a RNA-protein fusion (PROfusion™) using the technique developed by Szostak et al., U.S.S.N. 09/007,005, now U.S. Patent No. 6,258,558 B1, and U.S.S.N. 09/247,190, now U.S. Patent No. 6,261,804 B1; Szostak et al., WO98/31700; and Roberts & Szostak, Proc. Natl. Acad. Sci. USA (1997) vol. 94, p. 12297-12302. Briefly, the fragments were transcribed using an Ambion in vitro transcription kit,

MEGAshortscript (Ambion, Austin, TX), and the resulting mRNA was gel-purified and ligated to a DNA-puromycin linker using DNA ligase. The mRNA-DNA-puromycin molecule was then translated using the Ambion rabbit reticulocyte lysate-based translation kit. The resulting mRNA-DNA-puromycin-protein PROfusion™ was purified using Oligo(dT) cellulose, and a complementary DNA strand was synthesized using reverse transcriptase and the RT primers described above (Unisplint-S or flagASA), following the manufacturer's instructions.

Replace the first partial paragraph on page 27 (lines 1-3) with the following amended partial paragraph:

from the master library following the general procedure described in Szostak et al., U.S.S.N. 09/007,005, now U.S. Patent No. 6,258,558 B1, and 09/247,190, now U.S. Patent No. 6,261,804 B1; Szostak et al., WO98/31700; and Roberts & Szostak, Proc. Natl. Acad. Sci. USA (1997) vol. 94, p. 12297-12302 (Figure 8).

In the Claims

Amend claims 1 and 8-22 as follows.

1. (Amended) A library of scaffold-based proteins, [comprising a] wherein said scaffold is derived from the tenth module of the human fibronectin type III domain (^{10}Fn3) having at least [one] three randomized loops, said library comprising proteins being characterized by [its] their ability to bind to [a] compounds that [is] are not bound by [the corresponding naturally-occurring] said human fibronectin type III domain and wherein said binding ability results from said randomization of said at least three loops.

8. (Amended) The [protein] library of claim [4] 1, wherein said library comprises

proteins in which one of said [the second loop] randomized loops [of said ^{10}Fn3] is extended in length relative to [the naturally-occurring module] the corresponding loop of human ^{10}Fn3.

9. (Amended) The [protein] library of claim [4] 1, wherein said [^{10}Fn3] proteins lack[s] an integrin-binding motif.

10. (Amended) The [protein] library of claim 9, wherein said integrin-binding motif is replaced by an amino acid sequence comprising a basic amino acid-neutral amino acid-acidic amino acid motif.

11. (Amended) The [protein] library of claim [10] 9, wherein said integrin-binding motif is replaced by an amino acid sequence comprising serine-glycine-glutamate.

12. (Amended) The [protein] library of claim 1, wherein said proteins of said library lack[s] disulfide bonds.

13. (Amended) The [protein] library of claim 1, wherein said proteins of said library [is] are part of [a] fusion proteins.

14. (Amended) The [protein] library of claim 13, wherein said fusion proteins further comprise[s an] immunoglobulin F_c domains.

15. (Amended) The [protein] library of claim 13, wherein said fusion proteins further comprise[s a] complement proteins.

16. (Amended) The [protein] library of claim 13, wherein said fusion proteins further comprise[s a] toxin proteins.

17. (Amended) The [protein] library of claim 13, wherein said fusion proteins further comprise[s an] albumin proteins.

18. (Amended) The [protein] library of claim 1, wherein said proteins of said library are [is] covalently bound to [a] nucleic acids.

19. (Amended) The [protein] library of claim 18, wherein said nucleic acids encode[s] said proteins.

20. (Amended) The [protein] library of claim 18, wherein said nucleic acid is RNA.

21. (Amended) The [protein] library of claim 1, wherein said proteins of said library [is a] are multimers.

22. (Amended) The [protein] library of claim 1 or 9, wherein said proteins of said library [is] are formulated in a physiologically-acceptable carrier.

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